

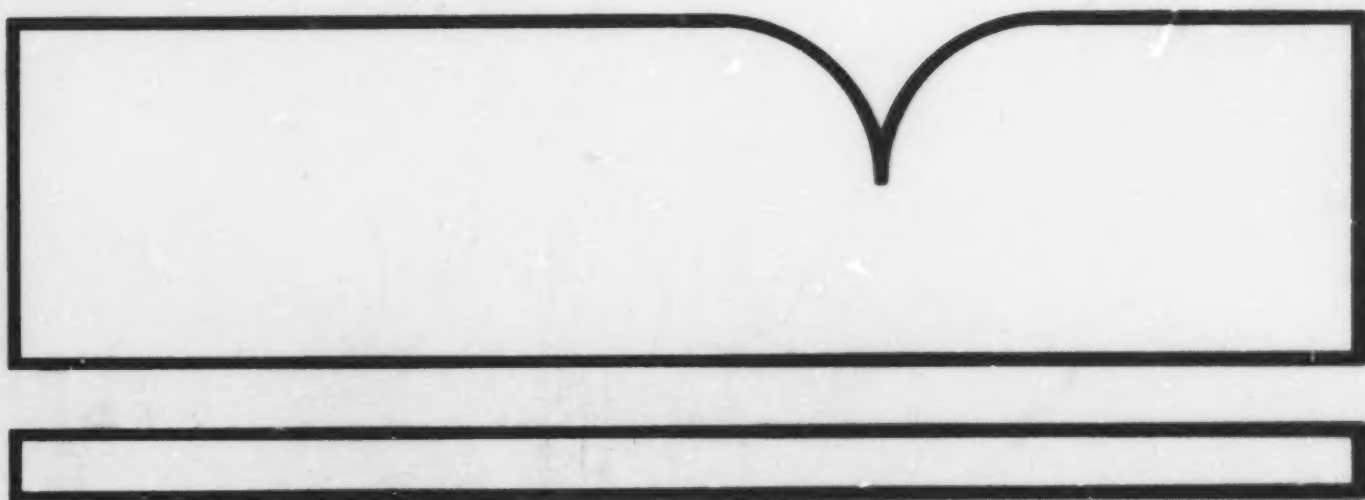
Evaluation of the Mutagenic
Potentials of Platinum Compounds

North Carolina Central Univ., Durham

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16. ABSTRACT Five platinum compounds, <u>cis-platinum diaminodichloride (cis-PDD)</u> , <u>trans-platinum diaminodichloride (trans-PDD)</u> , <u>platinum diaminodinitrite</u> , <u>platinum dichloride (PtCl₂)</u> , and <u>platinum tetrachloride (PtCl₄)</u> were evaluated for their mutagenic activity at the thymidine kinase locus in the mouse lymphoma cells line L5178Y. Three of these compounds, i.e., <u>cis-PDD</u> , <u>trans-PDD</u> and <u>PtCl₄</u> yielded significantly higher mutagenic frequencies as compared to the control. Two platinum compounds, i.e., <u>cis-PDD</u> and <u>PtCl₄</u> , were also judged to be mutagenic on the basis of results obtained in the <u>Phaseolus mungo</u> plant test system.		
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PLATINUM COMPOUNDS

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The chemical industry is the largest consumer of platinum. Platinum is used as a catalyst, especially for the production of fertilizers and explosives.

In the development processes, there are always opportunities for spillage, vapor entrainment or other mechanisms for the loss of platinum into the environment. It is, therefore, pertinent to determine the safety of these compounds for human health.

F. G. Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

Five platinum compounds, cis-platinum diaminodichloride (cis-PDD), trans-platinum diaminodichloride (trans-PDD), platinum diaminodinitrite, platinum dichloride (PtCl_2), and platinum tetrachloride (PtCl_4) were evaluated for their mutagenic activity at the thymidine kinase locus in the mouse lymphoma cells line L5178Y. Three of these compounds, i.e., cis-PDD, trans-PDD and PtCl_4 yielded significantly higher mutagenal frequencies as compared to the control. Two platinum compounds, i.e., cis-PPD and PtCl_4 , were also judged to be mutagenic on the basis of results obtained in the Phaseolus mungo plant test system.

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INTRODUCTION

It is generally agreed that man has always been exposed to toxic agents including potentially mutagenic substances naturally present in the environment. However, striking advances in technology have increased this exposure to a greater extent. Every year thousands of new chemicals are put into the market in the form of food additives, drugs, cosmetics, pesticides, herbicides, and industrial by-products. These chemicals present a possible health hazard to man. It has been demonstrated that a greater proportion of environmental chemical mutagens are also carcinogens (13), and that an impressive proportion of hospital admissions reflect genetic disabilities.

Platinum compounds are among the new chemicals recently introduced into the environment in the form of active components in catalytic converters in emission control devices of automobiles. Some of the platinum compounds are also being considered for use as anti-tumor agents.

Platinum metals and their salts are already being used in electrical, dental, medical, glass, jewelry and decorative industrial products. However, the chemical industry is the largest consumer of platinum. Platinum is used as a catalyst, especially for the production of nitric acid, which is used in tonnage quantities in the production of fertilizers and explosives. With the steady world-wide increase in population, as well as demand for food products, continued demand for fertilizers is expected for a long time.

In these processes, there are always opportunities for spillage, vapor entrainment or other mechanisms for loss of platinum into the environment. It is, therefore, pertinent to determine the safety of these compounds for human health.

In a series of reports, Rosenberg and his associates first demonstrated the toxicity of platinum compounds in bacteria (19-21) and then showed that some of these compounds induced inhibition and regression of solid tumors and leukemias (22,23) in experimental animals.

The high chemotherapeutic index, particularly of cis-platinum diamino-dichloride (Cis-PDD) has aroused great interest. Its effectiveness against several types of tumors has been reported (10,11,16). Recently, Pascoe and Roberts (16,17) have pointed out the similarity of reactions of platinum (II) compounds and alkylating agents and were able to demonstrate that Cis-platinum (II) compounds cross-linked DNA strands of HeLa cells in in vitro experiments. The cross-linking of DNA in Escherichia coli by Cis-PDD has also been demonstrated by Beck (1). Cis-PDD causes induction of temperate bacteriophages (18) and is exceptionally lethal to DNA repair deficient mutants of E. coli (1).

The above observations suggested the occurrence of direct or indirect interaction between Cis-PDD and DNA which might result in the induction of mutation. Recently, Cis-PDD has been shown to induce point mutations of base pair substitution type in E. coli (1), S. typhimurium (2,12); and at HGPRT locus in Chinese hamster ovary cells (15,24). Cis-PDD has also been found to induce chromosomal breaks in mammalian cells in culture (4). Recently the mutagenicity of $PtCl_4$ in a sex-linked recessive lethal test in Drosophila has been reported by Valencia (25).

The objective of this project was to evaluate the mutagenic effects of a number of platinum compounds in an in vitro test system using thymidine kinase forward mutation assay in mouse lymphoma cells and in an in vivo assay by employing Phaseolus mungo seed germination and seedling growth test.

MATERIALS AND METHODS

The platinum compounds used in this study were 99 percent pure and were obtained from ICN Life Sciences Group, 121 Express Street, Plainview, New York 11803. The Phaseolus mungo seeds were obtained from Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. The culture medium was obtained from Grand Island Biological Company, 3175 Staley Road, Grand Island, New York 14072.

A. TK Mouse Lymphoma Forward Mutation Assay

The mouse lymphoma cell line L5178Y, clone 3.7.2 was kindly provided by Dr. D. Clive of Genetic Toxicology Laboratory, Burroughs Wellcome Company, Research Triangle Park, North Carolina. The experimental procedure for mutagenesis at the thymidine kinase locus as developed by Dr. Clive and his associates (6,7,8) was employed in this study with minor modifications.

L5178Y mouse lymphoma cells have been found to be diploid at the thymidine kinase (TK) locus and selection media have been developed for isolating TK-competent (TK+/- and TK+/+) and TK-deficient (TK-/-) mutants arising at low frequencies among cells of the opposite phenotype.

The cells were grown in suspension culture in RPMI-1640 medium (R_0) supplemented with penicillin (50 units/ml), streptomycin (50 μ g/ml) and

horse serum 10 percent (R_{10}), with the addition of sodium pyruvate (100 mg/ml R_{10}), in a shaker incubator at 37°C. For daily maintenance, one volume of culture was diluted with four volumes of fresh R_{10p} . For weekend maintenance, one volume of culture was diluted with about 100 volumes of R_{10p} .

The freezing and thawing of the cells was done according to the procedures outlined by Clive and Spector (9).

Cell counting was accomplished by incubating cells with 0.1 percent trypsin in saline. One ml of cells were diluted with 9 ml trypsin and incubated at 37°C. for 10 minutes. After mixing them for a few seconds in a vortex mixer, the cells were counted using an electronic particle counter.

Experimental Procedure:

Cleansing the Cultures. TK competent heterozygote (TK+/-) cells grown in suspension cultures mutate to TK-deficient (TK-/-) at the rate of approximately 2×10^6 mutation/locus/generation (8). To induce the frequency of spontaneously accrued mutants as low as possible, the cultures were cleaned by growing them in a medium supplemented with thymidine, hypoxanthine, methotrexate and glycine (THMG). This selective medium permitted the normal growth of TK+/- while killing the TK-/-mutants.

The cleansing culture technique employed was as follows: On Wednesday, 0.5 ml THMG stock was added to 50-ml aliquot of cells. The next day, Thursday, between 5 and 10 ml of this culture was pelleted and resuspended in 300 ml of R_{10p} supplemented with 3 ml of thymine hypoxanthine guanine (THG). The cleansed culture became ready to use for experimental procedure.

Cleansed culture of TK+/- was diluted to a concentration of 0.3×10^6 particles/ml and thoroughly mixed. Then 50 ml aliquots were dispensed into 125 ml flasks and placed in a shaker incubator.

Mutagenesis: The test chemicals were dissolved either in saline or dimethylsulfoxide (DMSO). The platinum stock solutions were always freshly prepared and filtered through 0.22 μ m nalgene filters before use. Ethyl-methane sulfonate (EMS) at a concentration of 5 mM and 8 mM was used as a positive control and solvent and untreated cultures served as negative controls. In the initial stages 1,3,5 and 8 mM concentrations of EMS were used to establish the dose response curve.

The culture aliquots (50 ml, containing 0.3×10^6 cells/ml) were treated with the test compounds ranging from 1 μ M to 1300 μ M and incubated for four hours under normal growth conditions.

Mutagen removal: The cells were collected by centrifugation (500 X g, for 10 minutes) and the supernatant containing the non-incorporated mutagen was discarded. The cells were resuspended in 25 ml of fresh R_{10p} with gentle vortex mixing and pelleted as before. The supernatant was discarded and the washing step was repeated.

Expression days: The washed cells were quantitatively transferred into appropriately labeled 250 ml flasks containing 50 ml of R_{10p} and allowed to express their genetic lesions in the shaker incubator. The cell cultures were counted daily and diluted to 0.3×10^6 particles/ml on each of two expression days and on day three, the cloning day. The treated cultures, which showed over 90% killing during the expression period, were not used for cloning.

Each experiment was repeated at least twice, after an interval of two weeks, to confirm the results.

Cloning Day:

(a) Preparation. The stock solution Trifluorothymidine (TFT) was thawed and melted agar was added to the previously prepared cloning medium. Six sterile petri dishes per culture were labeled, 3 for viable count (VC) and 3 for "TFT."

(b) Cloning. 10 ml of each culture at 0.3×10^6 particles/ml or a total of 3×10^6 were centrifuged (500 X g for 10 minutes) and the cells were collected and resuspended in about 1 ml of retained supernatant. The cell suspensions were transferred to 250 ml flasks containing 100 ml of cloning medium (labeled "TFT") and were mixed in a shaker incubator for 30 minutes (concentration 3×10^4 particles/ml).

Aliquotes of 0.5 ml of the TFT suspensions were then diluted by adding to 50 ml of cloning medium in 125 ml flasks ($1:100$; 3×10^2 particles/ml) labeled "diluted cultures." One ml of TFT stock was added to TFT flask cultures.

The "diluted cultures" and TFT flasks were placed in a shaker incubator. After 15 minutes the "diluted cultures" were further diluted by taking 1 ml and adding to 100 ml cloning medium in 250 ml flasks labeled "VC" and TFT flasks were removed and 33 ml of cultures were poured into appropriately labeled sterilized petri dishes. The plates were geled at -20°C . for 12 minutes, warmed at room temperature for 5 minutes and incubated at 37°C . for 10 days. The colonies were counted using an electronic colony counter and results were calculated as follows:

(1) GROWTH: Daily growth rate of cells was determined by dividing final concentration after 24 hours by initial or starting concentration.

$$\text{GROWTH (24 hours)} = \frac{\text{Final concentration}}{\text{Initial concentration}}$$

(2) CUMULATIVE GROWTH RATE: The cumulative growth reflects the total growth of cells for the 3-day expression period after treatment with chemicals. It was obtained by multiplying final concentration for 24 hours by growth of 48 hours which gave cumulative growth for 48 hours; this was further multiplied by growth for 72 hours.

$$\begin{aligned} \text{CUMULATIVE GROWTH RATE} &= \text{Final concentration} \\ &\quad \text{growth 24 hours} \\ &\quad \times \text{growth 48 hours} \times \\ &\quad \text{growth 72 hours} \end{aligned}$$

(3) PERCENT CELL SURVIVAL IN SUSPENSION: Percent cell survival in suspension was determined by dividing cumulative growth for 72 hours of treated cultures by cumulative growth for control (without treatment) and multiplying by 100, where control is assumed to be 100 percent survival.

$$\begin{aligned} \text{PERCENT CELL SURVIVAL IN SUSPENSION} \\ &= \frac{\text{Cumulative growth of treated culture}}{\text{Cumulative growth of control}} \times 100 \end{aligned}$$

(4) CLONING EFFICIENCY (C.E.): It was determined by multiplying VC of treated cultures X 100 and divided by VC of control.

$$\text{C.E.} = \frac{\text{VC of treated culture}}{\text{VC of control culture}} \times 100$$

(5) PERCENT GROWTH: Percent growth was determined by multiplying C.E. by cell survival in suspension and divided by 100.

$$\text{PERCENT GROWTH} = \frac{\text{C.E.} \times \text{percent cell survival in suspension}}{100}$$

(6) SPONTANEOUS MUTATION FREQUENCY (f): This was determined by dividing average of TFT colonies by average of VC for the cultures untreated.

$$f = \frac{\text{Average TFT}}{\text{Average VC}}$$

(7) INDUCED MUTATION FREQUENCY (fi): This was obtained by subtracting the spontaneous mutation frequency for each treated culture from the mutation frequency of the control.

$$f_i = \text{Mutation Frequency of treated culture minus mutation frequency of control}$$

Induced mutation frequency greater than twice the spontaneous mutation frequency was considered as a positive response.

B. Phaseolus Mungo Seed Germination and Seedling Studies

Two of the platinum compounds, i.e., cis-PDD and PtCl_4 , were tested in the plant seed germination test in order to verify the results obtained in the mammalian cell culture systems.

Twenty-five seeds of P. mungo were treated in dark at specific concentrations for 4 hours in 10 mls of freshly prepared solution of the test compounds. The seeds intended for control were kept in the distilled water for 4 hours. After treatment the contents of each vial were placed on a filter paper in a funnel where they were washed for 15 minutes in distilled

water. After washing, the seeds were planted in the greenhouse. The data on the germination was recorded on the sixth day and the twelfth day after planting. The growth of the seedlings of the control and treated cultures was recorded on the twentieth day after planting.

RESULTS

The dose-dependent response by EMS in terms of cytotoxicity as measured by growth in suspension and percent growth, as well as induced mutation frequencies, is given in Figure 1. These results are in general agreement with those reported by Clive (8) for this test system.

After establishing the reliability of the test with a known positive control compound, the various platinum compounds were tested for mutagenicity in this test system. Figure 2 shows the cytotoxic and mutagenic effects obtained after exposing the cells to various concentrations of cis-PDD. This compound was highly toxic to the mouse lymphoma cells. At 10 μM concentration the growth of cells was reduced to 4.3 percent and cell survival in suspension was reduced to 13 percent. The very high induced mutation frequencies at 25 μM -dose may be misleading due to very low growth of the treated cultures. However, even with 31.7 percent growth and 44 percent survival at 5 μM treatment level, the induced mutation frequencies (142×10^{-6}) were more than twice the spontaneous rate (58×10^{-6}). These observations are in agreement with those obtained by O'Neill et al. (15) and Taylor (24) in CHO cells, Lecointe et al. (12) in S. typhimurium, and Benedict et al. (2) in E. coli.

Table 1
Cytotoxic and Mutagenic Effects of Ethyl Methanesulfonate (EMS) in TK Locus in Mouse Lymphoma Cells

Treatment	24 hours growth ($\times 10^6$)	48 hours growth ($\times 10^6$)	72 hours growth ($\times 10^6$)	Percent survival in suspension	Percent growth	Mutation frequency (10^{-6})	Induced Mut. Freq. (10^{-6})
0	5.3	10.8	60.1	100.0	100.0	58	-
1 mM	3.2	2.0	5.1	77	60.5	314	256
3 mM	1.9	1.4	3.4	51	30.7	518	460
5 mM	2.8	10.0	50.1	83.0	9.4	1094	1036
8 mM	2.6	4.6	17.4	29.0	3.6	1555	1497

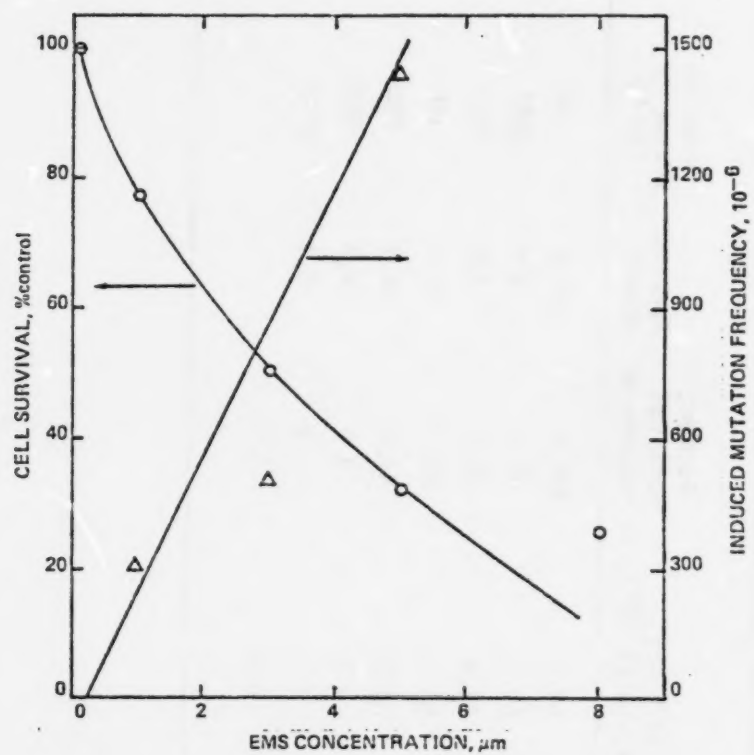


Figure 1. Effect of Ethyl methanesulphonate on cell survival (percent of the control) for 3-day expression period and induced mutation frequency.

Table 2
Effects of Cis-Platinum Diamminodichloride [cis-Pt (NH₃)₂Cl₂] on the Growth
Rate and Induced Mutation Frequency (TK+/-TK-/-) in Mouse Lymphoma Cells

Treatment	24 hours growth (X 10 ⁶)	48 hours growth (X 10 ⁶)	72 hours growth (X 10 ⁶)	Percent survival in suspension	Percent growth	Mutation frequency (10 ⁶)	Induced Mut. Freq. (10 ⁶)
0	5.3	10.8	60.1	100.0	100.0	58	-
5 mM EMS	2.8	10.0	50.1	83.0	9.4	1094	1036
8 mM	2.6	4.6	17.4	29.0	3.6	1555	1497
1 μ M <u>Cis-PDD</u>	4.7	10.0	50.1	83.0	51.5	150	92
5 μ M	3.2	6.2	26.5	44.0	31.7	200	142
10 μ M	2.5	3.0	7.8	13.0	4.3	860	802
25 μ M	2.3	1.2	1.1	1.8	0.1	2746	2688
50 μ M	1.6	0.6	0.6	0.9	-	-	-

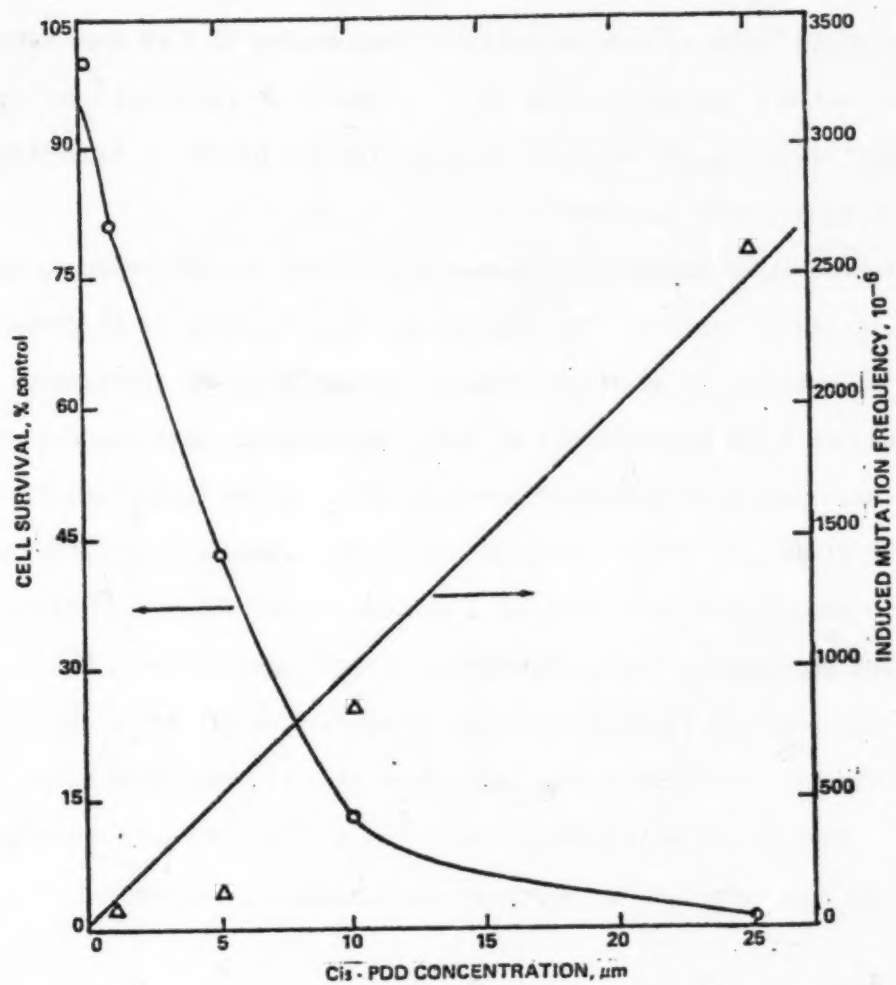


Figure 2. Effect of Cis-Platinum diaminodichloride (Cis-PDD) on cell survival in suspension (percent of the control) for 3-day expression period and induced mutation frequency.

However, contrary to the previously published reports, trans-PDD in our experiments showed highly toxic and mutagenic response. As shown in Figure 3 and Table 3, induced mutation frequencies at 1 μ M dose were approximately 4 times the spontaneous rate. Although we could not confirm it, the positive mutagenic response of trans-PDD may be due to impurities present in the test compound.

The positive mutagenic response by platinum tetrachloride is shown in Figure 4 and in Table 4. The induced mutation frequencies at doses ranging from 25 μ M to 150 μ M are significantly greater than the spontaneous mutation frequencies. The mutagenicity of PtCl_4 has recently been reported in the sex-linked recessive test in *Drosophila* (25). Using human lung fibroblasts (Strain WI-38) and rabbit alveolar macrophages, Waters *et al.* (26) were able to demonstrate that PtCl_4 at a concentration of 0.007 mM causes significant decrease in the incorporation of C^{14} -thymidine into DNA.

The negative mutagenic response of *pt* diamino dinitrite and platinum dichloride in this test system is evident from Figures 5 and 6 and Tables 5 and 6. In both cases the induced mutation frequencies were not significantly different from the spontaneous mutation frequencies.

Table 3
Effects of Trans-Platinum Diaminodichloride [Trans-Pt (NH₃)₂Cl₂] on the Growth
Rate and Induced Mutation Frequency (TK+/-TK-/-) in Mouse Lymphoma Cells

Treatment	24 hours growth (X 10 ⁶)	48 hours growth (X 10 ⁶)	72 hours growth (X 10 ⁶)	Percent survival in suspension	Percent growth	Mutation frequency (10 ⁶)	Induced Mut. Freq. (10 ⁶)
0	5.7	10.7	50.3	100	100	53	-
1 µM	3.3	4.7	15.3	31.0	8.7	265	212
3 µM	3.0	2.3	5.3	10.0	3.7	486	433
5 µM	2.3	1.3	1.3	2.3	0.19	618	565
10 µM	1.7	0.8	0.7	1.4	-	-	-
5 mM EMS	5.6	5.6	26.3	69.0	40.0	1013	914

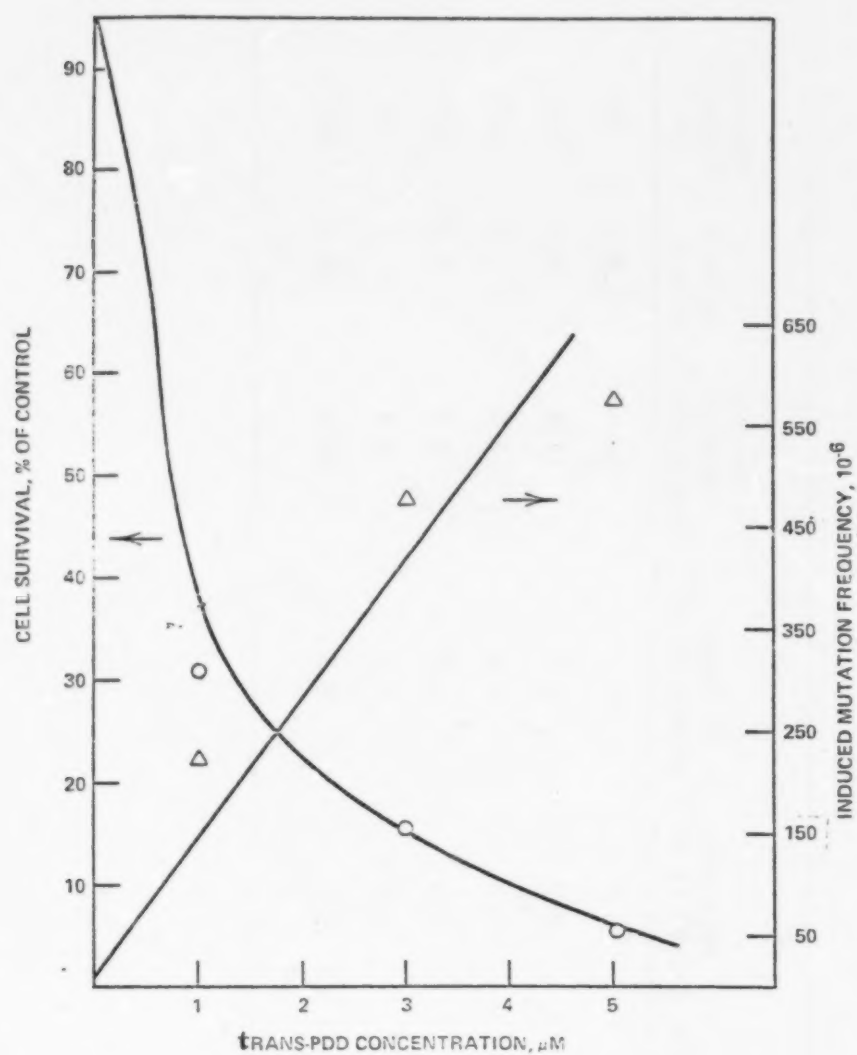


Figure 3. Effect of trans-PDD on cell survival in suspension for three days expression period and induced mutation frequency.

Table 4
Effects of Platinum Tetrachloride (PtCl₄) on the Growth Rate
and Induced Mutation Frequency (TK⁺/TK⁻) in Mouse Lymphoma Cells

Treatment (μ M)	24 hours growth	48 hours growth	72 hours growth	Percent survival	Cloning efficiency	Percent growth	Mutation frequency (10 ⁻⁶)	Induced mutation frequency (10 ⁻⁶)
Control	5.7	10.2	51	100	100	100	38	
50	3.7	5.8	33	65	75	49	214	176
75	3.1	4.7	25	49	48	28	310	272
100	2.3	2.8	11	22	39	9	399	361

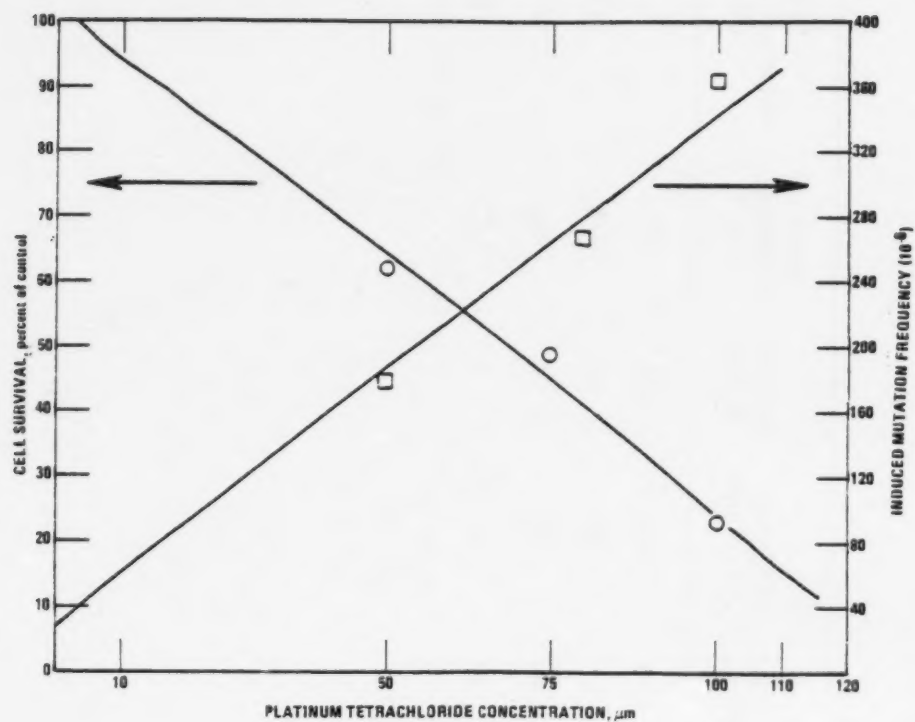


Figure 4. Effects of platinum tetrachloride on cell survival in suspension for the three days expression period and induced mutation frequency.

Table 5
Effects of Platinum Diaminodinitrite [(Pt (NH₃)₂(NO₂)] on the Growth
Rate and Induced Mutation Frequency (TK+/-TK-/-) on Mouse Lymphoma Cells

Treatments	24 hours growth	Accumulative growth 48 hours growth (X 10 ⁶)	72 hours growth (X 10 ⁶)	Percent survival in suspension	Percent growth	Mutation frequency (10 ⁶)	Induced Mut. Freq. (10 ⁶)
0	5.2	9.4	53.9	100	100.0	50	-
5 mM EMS	2.8	10.0	50.1	93	9.4	1094	1044
8 mM EMS	2.6	4.6	17.4	32	3.6	1555	1505
1 μ M <u>Cis-PDD</u>	4.9	9.8	52.5	97	107.0	54	4
5 μ M	5.2	9.7	53.4	99	91.5	74	24
10 μ M	5.1	10.5	51.5	96	84.0	70	20
50 μ M	2.7	2.8	11.3	21	16.2	63	13
100 μ M	1.5	0.6	1.0	1.85	1.3	40	-
200 μ M	1.4	0.3	0.3	0.5	-	-	-

Table 6
Effects of Platinum Dichloride on the Growth Rate
and Induced Mutation Frequency (TK+/-TK-/-) in Mouse Lymphoma Cells

Treatment	24 hours growth (X 10 ⁶)	48 hours growth (X 10 ⁶)	72 hours growth (X 10 ⁶)	Percent survival	Percent growth	Mutation freq. (10 ⁻⁶)	Induced Mut. Freq. (X 10 ⁻⁶)
0	6.3	8.9	38.3	100	100	99	-
5 mM EMS	5.0	5.6	26.3	69	40	1013	914
50 μ M	6.7	8.6	43.0	112	97	105	6
100 μ M	6/3	7.6	35.7	93	81	122	23
200 μ M	6.0	5.9	25.4	66	84	52	0 (-47)
500 μ M	5.3	5.0	30.0	78	79	85	0 (-14)
800 μ M	4.0	2.9	18.3	48	47	92	0 (-7)

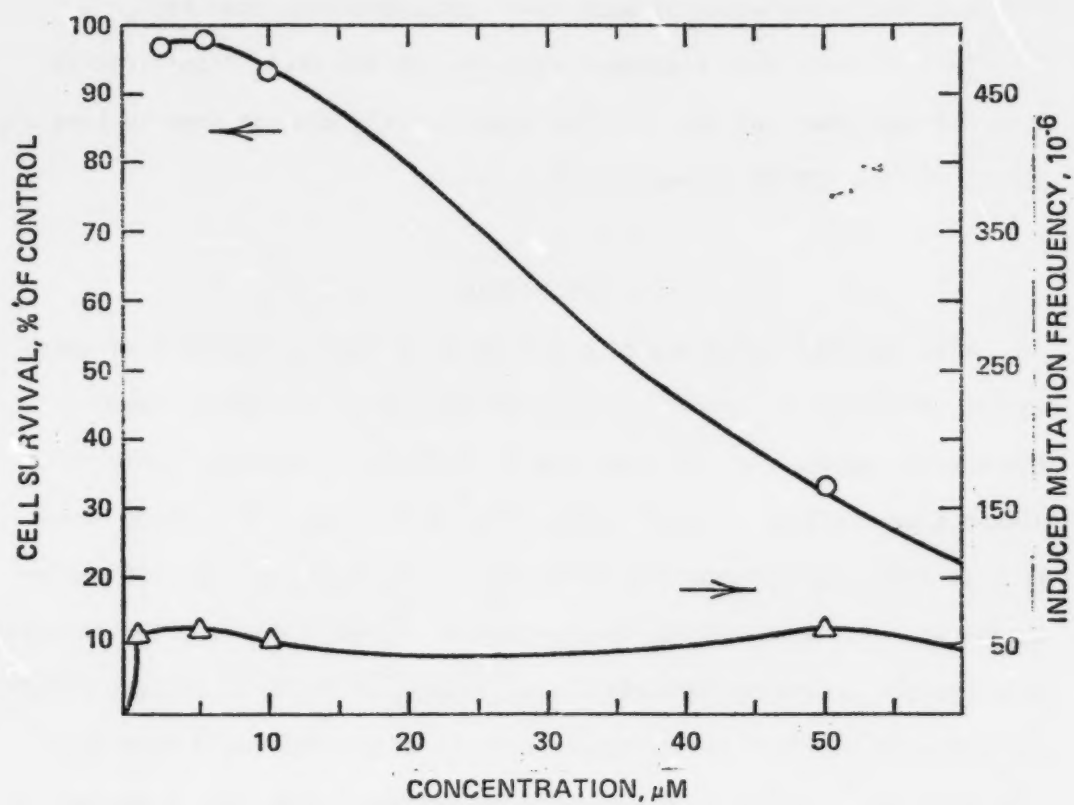


Figure 5. Effects of Platinum diaminodinitrite on cell survival in suspension for the three day expression period and induced mutation frequency.

The data presented in Table 7 clearly establishes that treatment with cis-PDD and platinum tetrachloride retarded seed germination and the degree of retardation was dose dependent. It was further noted that cis-PDD affected seed germination at much lower concentrations than PtCl_4 .

Table 8 shows that treatment with cis-PDD and PtCl_4 significantly retarded the growth of the seedling whereas PtCl_2 did not seem to have any affect even at 800 mM concentration.

DISCUSSION

With the increasing use of platinum as an active component of automotive catalytic converters, or through its use as antitumor chemotherapeutic agents and its heavy use in fertilizer industry, there is always a possibility of human exposure to this metal. This study shows that at least certain platinum compounds, i.e., PtCl_4 and cis-PDD may be mutagenic. The mutagenicity of cis-PDD has already been reported in several test systems including bacteria (12,12) mammalian cells in culture (15,24) and *Drosophila* (25). The mutagenic effects of cis-PDD could have been predicted on the bases of the earlier observations that this compound can form interstrand cross-links in bacterial DNA (11). It is, however, not clear which type of structures in the double stranded DNA are, in fact, cross-linked by platinum compounds. It is possible that platinum compounds may also bind monofunctionally to DNA.

Table 7
Percent Germination of *Phaseolus mungo* Seeds
Treated with Platinum Compounds

Treatment dose (in mM)	Germination	
	6 Days	12 Days
Control	91	95
<u>Platinum Tetrachloride</u>		
25	60	93
50	14	73
75	3	69
100	3	51
150	1	10
<u>Cis-PDD</u>		
1	66	89
5	67	80
10	11	73
25	2	63
50	1	41
<u>PtCl₂</u>		
50	90	91
100	67	80
200	93	93
500	91	94
800	80	89

Table 8
Size of Seedlings Scored from 1-10 (10 largest)
25 Days after Treatment with Platinum Compounds

Treatment dose (in mM)	Seedling size
Control	10
<u>PtCl₄</u>	
25	7.0
50	4.0
75	3.5
100	2.0
150	1.0
<u>Cis-PDD</u>	
1	6.0
5	6.5
10	3.0
25	3.0
50	2.1
<u>PtCl₂</u>	
50	10.0
100	9.0
200	9.5
500	8.5
800	10.0

Although the mutagenesis tests based on mammalian cells in culture are not sufficiently developed to illustrate the mechanism of mutation involved, the Salmonella tests (2,12) have clearly established that cis-PDD caused mutations are of base pair substitution type.

The data from Phaseolus reported in this study provides only an indirect evidence of mutagenicity of platinum compounds. Retardation of seed germination and seedling growth have been considered synonymous with seedling injury following irradiation of seed (5). Generally, such retardation or injury is considered to be indicative of genetic change induced in the meristematic cells of the seed. Also, there has been repeatedly demonstrated a positive correlation between the degree of seedling injury and amount of genetic injury resulting from different types of radiation (6).

Cis-PDD, which is reported to be an antitumor agent, was more potent as a mutagen in both the test systems than other platinum compounds. This further supports the concept that most of the chemotherapeutic agents may also be potent mutagens in addition to being powerful toxicants.

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